Haemolytic Disease of the Newborn Caused by Anti-Lan Antibody

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Summary: The first recorded example of anti-Lan associated with haemolytic disease of the newborn is reported. This emphasizes the importance of screening for atypical antibodies early in pregnancy, even though prophylactic use of anti-D immunoglobulin will eventually reduce the incidence of haemolytic disease due to anti-D antibody.

Introduction

Haemolytic disease of the newborn due to anti-D antibody in Rh-negative women will become progressively less frequent owing to the prophylactic use of anti-D immunoglobulin. Nevertheless, cases of jaundice of the newborn will continue to appear, since haemolytic disease of the newborn can be caused by a number of different antibodies, other than anti-D. It will still be important, therefore, to continue the examination of blood samples during a pregnancy for the presence of other atypical antibodies, particularly in cases where there is a previous history of blood transfusion of the mother, where there is a history of abortion and/or stillbirth, or where the mother has previously given birth to jaundiced infants.

This paper describes the first case of haemolytic disease of the newborn due to anti-Lan, an antibody directed against a high-frequency antigen and, in this patient, probably evoked by the previous blood transfusion.

Case History

The patient, a 26-year-old Caucasian woman, had one full-term, previously healthy, child born in 1965. At this birth a forceps delivery was undertaken because of foetal distress, and five days later, following a severe secondary haemorrhage, she was transfused with 3 pints (1·7 litres) of compatible whole blood. The patient was group O, Rh-positive, and during the 14th week of her second pregnancy a sample was submitted for testing to the Blood Transfusion Service in Manchester and was found to contain an anti-Lan antibody.

During her pregnancy she was treated with oral iron and folic acid, and at 36 weeks a "total dose" (25 ml.) of Imferon drip was given, but despite this, when she was admitted for delivery at term her haemoglobin concentration was 10 g./100 ml. In view of her general clinical condition and her previous obstetric history it was decided to transfuse her with the stored frozen compatible blood from her brother, the only Lan-negative compatible blood available, which was thawed and deglycerolized by means of the Huggins cyto-agglomerator. This was returned from the Blood Group Reference Laboratory and was given with beneficial results. The transfusion was uneventful and the following morning she was induced with buccal Pitocin.

The patient had a normal delivery of a male infant weighing 8 lb. 8 oz. (3,855 g.), after a labour of 2 hours 45 minutes. Intravenous Syntometrine 0·5 mg. was given with delivery of the anterior shoulder. The placenta and membranes were delivered by the Brandt–Andrews manœuvre and the estimated blood loss was 6 oz. (170 ml.). After delivery the infant's clinical condition was satisfactory, and the cord blood findings were: total serum bilirubin 2·6 mg./100 ml., direct reacting bilirubin 0·8 mg./100 ml., reticulocytes 8%, and total white cell count 23,100/cu. mm. The stained blood film showed two nucleated red cells per 100 white blood cells.

The direct antiglobulin test on the cord red cells was positive (+++) with a broad-spectrum antiglobulin reagent, but tests with specific antiglobulin reagents showed that the cells reacted only with an anti-complement reagent. Anti-Lan of 1 in 4 titre was shown to be present in the cord serum. Subsequent progress of the infant is shown in Fig. 1.

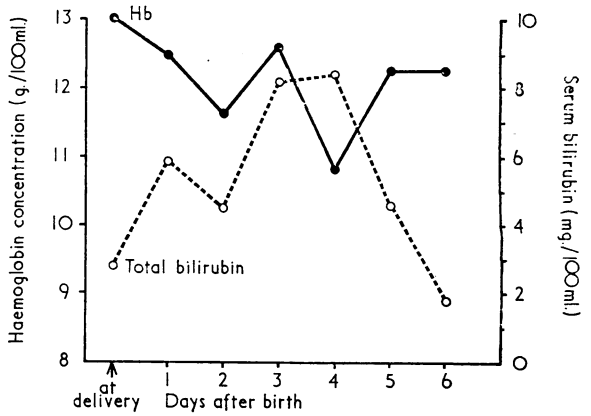

FIG. 1.—Haemoglobin concentration and serum bilirubin of infant.

Four weeks after delivery the haemoglobin concentration was 9 g./100 ml., and 10 weeks after delivery it was 10·8 g./100 ml., and the infant was thriving well.

Materials and Methods

Standard serological methods were used (Stratton and Renton, 1958), and the immunoglobulin separation techniques were those of Stratton, Gunson, and Rawlinson (1962) and Stratton, Smith, and Rawlinson (1968).

Serological Investigations

Preliminary investigations showed the patient to be group O, Rh-positive, and her serum was found to contain an antibody, which reacted with all of a panel of "fully-typed" group O cells. In addition, the antibody reacted with all blood samples taken from 2,268 group O donors. The antibody did not react with her own cells, and later it was found to be negative with her brother's red cells.

Strong reactions were obtained in the indirect antiglobulin test, though no agglutination was observed against saline suspensions of cells at 16 or 37° C., serum albumin suspensions of cells at 37° C., and papain-treated cells on slides at room temperature.

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Although negative results were initially obtained with papain and bromelin techniques, it was found that serum treated with a quarter volume of M/20 Na_2.E.D.T.A. (*p*H 8·0) gave good agglutination with papain-treated cells in tubes at 37° C. Serum modified in this way was used to screen the 2,268 donors, papain-treated cells in Chown's capillary tubes being used. No negative samples were found.

Powerful reactions were obtained when the antibody was tested against papain-treated cells with the anti-human globulin technique. It has also been shown that enhanced reactions are obtained by pretreating red cells with ficin and examining them by means of a two-stage complement-binding technique (Polley and Mollison, 1961). It seems evident, therefore, that the antigen is not destroyed by enzymes, though antibody activity against enzyme-treated cells is not readily apparent (van der Hart, Moes, Veer, and van Loghem, 1961).

The patient's own cells were grouped as follows: O, R_1r, MSNs, P_1, Lu(a−), K−, Kp(a−), Le(a−b+), Fy(a+), Jk(a−b+), Sd(a+), I+, Bu(a−), Vw−, Mg−, He−, Wr(a−).

Work carried out at the Blood Group Reference Laboratory showed that the patient's cells possessed the following high-frequency antigens: H, U, P, Lu^b, Lu^aLu^b, k, Kp^b, Js^b, Ku, Yt^a, Co^a, Ve, Ge, Sm, LW, Di^b, Go^b, Gy^a, and Chido as well as reacting with five other unpublished high-frequency antisera. In addition, the cells failed to react with sera containing antibodies to the following rare antigens: C^w, C^x, Mi^a, Vw, Hut, Mur, Hil, Mg, Ny^a, St^a, Ri^a, Tm, Cl^a, Kp^a, Js^a, Yt^b, Bu^a, Di^a, Go^a, Bi^a, Be^a, Wr^a, Sw^a, Tr^a, By, Wb, Good, Kamhuber, and nine other unpublished antigens.

The patient's serum was found to react with cells of the following rare or infrequent types: O_h, R′R′, R″R″, D−−/D−−, V+VS+, S^uS^u, SS(s−), Lu(a−b−), KK, Kp(a+b+), Js(a+b+), K^oK^o, Le(a−b−), Fy(a−b−), Jk(a−b−), Yt(a+b+), Au(a−), Cs(a−), Co(a−), Sd(a−), Ve(a−), Ge(a−), Bu(a+), Sm(−), Luke(−), Chido(−), as well as with the cells of four donors lacking high-frequency antigens of unknown specificity.

A sample of Lan-negative cells was sent to the Manchester Regional Transfusion Centre by Professor J. J. van Loghem in Amsterdam, and on testing the patient's serum with these cells only weak results were obtained, similar to an AB serum control test. As Lan was the most likely established high-frequency antigen not previously accounted for, samples of the patient's and her brother's cells were sent to the Blood Group Reference Laboratory, where they were shown to be negative with anti-Lan.

Red cells and serum from the patient were sent to Professor van Loghem, who was able to confirm that the cells were Lan-negative and that the serum contained anti-Lan.

A dosage effect was not readily observed and the antibody had a titre of 1 in 16 with the majority of cells in the indirect antiglobulin test. The antibody was easily removed in a single absorption, but elution carried out by the method of Landsteiner and Miller (1925) failed to yield an active eluate. The antigen-antibody reaction was not inhibited either by saliva or hydatid cyst fluid.

Nature of Antibody

Papain-treated cells and untreated cells were incubated with the mother's antibody in the presence and also in the absence of complement. The reactions of the antibody-coated cells with specific antiglobulin reagents are shown in Table I. It can be seen that the antibody is a complement-fixing γG immunoglobulin. Table I also shows the reactions of specific antiglobulin sera, with antibody-coated cells sensitized with the cord serum.

TABLE I.—*Nature of the Antibody (as demonstrated by testing sensitized cells with specific antiglobulin reagents)*

	Antiglobulin Reagents				
	Broad Spectrum	Specific Reagents			Saline Control
		Anti-γG	Anti-γM	Anti-C′	
Maternal serum with:					
Untreated cells in the presence of complement	+ + + + +	+	−	+ + + + +	−
Untreated cells in the absence of complement	+ +	+	−	−	−
Papain-treated cells in the presence of complement	+ + + + +	+ + +	−	+ + + + +	−
Papain-treated cells in the absence of complement	+ + + + +	+ + + +	−	−	−
Papain-treated cells with γG—containing fractions, following D.E.A.E.-cellulose chromatography. Fresh serum added as a source of complement	+ + + +	+ + +	−	+ + + + +	−
Cord serum with:					
Untreated cells in the presence of complement	+ + + +	+	−	+ + + +	−
Untreated cells in the absence of complement	+	+	−	−	−
Papain-treated cells in the absence of complement	+ +	+ + +	−	−	−
Cord cells: Direct antiglobulin test	+ + +	−	−	+ + + +	−

The nature of the antibody was confirmed by separating the maternal serum on a Sephadex G-200 column, when antibody activity was located in the second (7S) peak, which contains γG immunoglobulin. It is evident that the antibody was a 7S γG globulin and would therefore be expected to cross the placenta. When the cord serum was tested anti-Lan was detected, and a summary of the type of reaction obtained with Lan-positive cells is shown in Table I. The antibody was also found in the fractions which contained γG protein, following column chromatography on D.E.A.E.-cellulose.

Family Studies

Table II shows the results of all grouping tests on the patient's relatives, and Fig. 2 gives the pedigree. It can be seen that only the propositus (II 3) and her brother (II 2) were found to be Lan-negative, and in the absence of negative results in the screening tests the brother proved to be the only compatible donor available. In view of this and the past history of the patient, a full donation was taken from him three months before the date of the patient's delivery. The blood was sent to the Army Blood Transfusion Service, Aldershot, where it was frozen by liquid nitrogen.

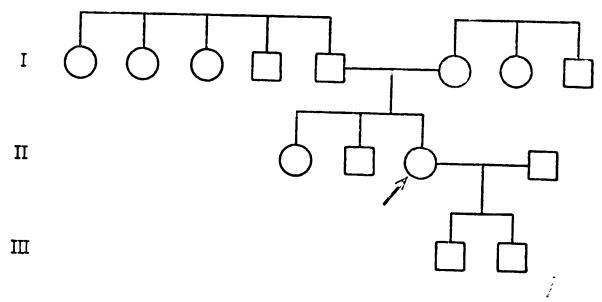

FIG. 2.—Family tree.

We confirmed van Loghem's findings that the Lan antigen is not closely linked with MNSs and Kidd, and in addition our family studies showed segregation from the sex chromosome and Kell blood group system.

TABLE II.—*Full Blood Groups of the Family (see Fig. 2)*

	ABO	Rh	MNS	P_1	Lu^a	K	k	Le^a	Le^b	Fy^a	Jk^a	Jk^b	Sd^a	Lan
I 1	O	R_1r	MMS	w	−	+		−	−	−	+			+
I 2	O	rr	MMS	+w	−	+		−	+	−	+			+
I 3	O	R_1r	MNS	+	−	−		−	+	−	+			+
I 4	O	R_1r	MMS	+	−	+		−	+	+	+			+
I 5	O	R_1R_2	MSMs	+	−	+	+	+	−	+	+			+
I 6	O	$R_2{}^u r$	MsNs	−	−	−	+	−	+	+	+			+
I 7	O	rr	MsNs	−	−	−		−	+	+	+			+
I 8	B	rr	MsNs	w	−	−		−	+	+	+			NT
II 1	O	R_1R_2	MSNs	+w	−	−	+	−	+	+	+			+
II 2	O	R_1r	MsNs	w	−	−		+	−	+	+		+	−
II 3	O	R_1r	MSNs	w+	−	−	+	−	+	+	−	+	+w	−
II 4	O	R_1R_1	NsNs	−	−	+		+	−	+	+			+
III 1	O	R_1r	MNS	w	−	+		−	+	+	+			+
III 2	O	R_1R_1												+

There was no evidence of retinitis pigmentosa in our patient or consanguinity in the family, as was encountered by van Loghem in his study of the Lan family.

Discussion

The first example of the antibody which defines the high-frequency antigen " Lan " was described by van der Hart, Moes, Veer, and van Loghem (1961). It was identified following a transfusion reaction in a 64-year-old man who had previously been transfused uneventfully.

A further example has recently been described by Grindon, McGinniss, Issitt, Reihart, and Allen (1968) in New York. We believe this is the third occasion on which anti-Lan has been found, and it is certainly the first recorded example of the antibody associated with haemolytic disease of the newborn.

The case emphasizes the importance of testing sera from previously transfused women early in pregnancy, so that blood group antibodies may be identified and their immunoglobulin types determined. The Lan antigen is of extremely high frequency, and we were fortunate to discover the antibody early in pregnancy. This enabled us to ensure that compatible blood was available for delivery while providing an opportunity for studying the effects of the antibody on the foetus.

Stratton and Renton (1967) stated that all Rh-negative women, and women who have had previous transfusions, or anaemic or jaundiced infants, or who have unexplained still-births or abortions late in pregnancy, should have their sera examined for atypical antibodies. In addition, all women of African or Asian descent resident in this country should have their blood examined because of the prevalence of Lewis antibodies.

The fact that the antibody was directed against a high-frequency antigen considerably diminished the chance of finding a compatible donor in the random screening tests, and in cases such as this considerable emphasis must be placed on family investigations. In this particular instance only the brother of the propositus was compatible with the anti-Lan serum, and it was of great value to be able to freeze and store his blood at an early stage in pregnancy. Consequently, at delivery, a pint (570 ml.) of stored blood was available for the patient, while, if required, a further fresh pint suitable for either the patient or her infant could still be obtained from the brother.

As anticipated the γG antibody was transferred across the placenta ; the baby had mild haemolytic disease of the newborn but fortunately no treatment was required.

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